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<b>(54) Title:</b> COMBINED VACCINE COMPOSITIONS			
<b>(57) Abstract</b> <p>Novel combined vaccine compositions preferentially for administration to adolescents are provided, comprising a hepatitis B viral antigen and a herpes simplex viral antigen and optionally in addition one or more of the following: an EBV antigen, a hepatitis A antigen or inactivated attenuated virus, an HPV antigen, a VZV antigen, an HCMV antigen, a Toxoplasma gondii antigen. The vaccine compositions are formulated with an adjuvant which is a preferential stimulator of TH1 cell response such as 3D-MPL and QS21.</p>			

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## COMBINED VACCINE COMPOSITIONS

This invention relates to novel vaccine formulations, methods for preparing them and their use in therapy. In particular the present invention relates to combination  
5 vaccines for administration to adolescents.

HSV-2 is the primary etiological agent of herpes genitalis. HSV-2 and HSV-1 (the causative agent of herpes labialis) are characterised by their ability to induce both acute diseases and to establish a latent infection, primarily in neuronal ganglia cells.  
10

Genital herpes is estimated to occur in about 5 million people in the U.S.A. alone with 500,000 clinical cases recorded every year (primary and recurrent infection). Primary infection typically occurs after puberty and is characterised by the localised appearance of painful skin lesions, which persist for a period of between 2 to 3  
15 weeks. Within the following six months after primary infection 50% of patients will experience a recurrence of the disease. About 25% of patients may experience between 10-15 recurrent episodes of the disease each year. In immunocompromised patients the incidence of high frequency recurrence is statistically higher than in the normal patient population.

20 Both HSV-1 and HSV-2 virus have a number of glycoprotein components located on the surface of the virus. These are known as gB, gC, gD and gE etc.

Vaccines for the prophylaxis of hepatitis B infections, comprising one or more  
25 hepatitis B antigens, are well known. For example the vaccine Engerix-B (Trade Mark) from SmithKline Beecham Biologicals is used to prevent Hepatitis B. This vaccine comprises hepatitis B surface antigen (specifically the 226 amino acid S-antigen described in Harford et. al. in Postgraduate Medical Journal, 1987, 63 (Suppl. 2), p65-70) and is formulated using aluminium hydroxide as adjuvant.

30

There is a need for effective combination vaccines to prevent diseases to which adolescents are particularly prone.

The present invention provides a vaccine composition comprising:

- (a) a hepatitis B viral (HBV) antigen; and
  - 5 (b) a herpes simplex viral (HSV) antigen
- in combination with an adjuvant which is a preferential stimulator of TH1 cell response.

The vaccine composition of the invention is of great benefit for administration to  
10 adolescents who may be particularly at risk of HBV, and/or HSV infection.

Optionally the vaccine composition of the invention additionally comprises one or more of a number of other antigens as described below.

15 It has been found that the vaccine compositions according to the invention surprisingly show no interference, that is to say that the immune response to each antigen in the composition of the invention is essentially the same as that which is obtained by each antigen given individually in conjunction with an adjuvant which is a preferential stimulator of TH1 cell response.

20

The vaccine Havrix (Trade Mark), also from SmithKline Beecham Biologicals is an example of a vaccine that can be used to prevent hepatitis A infections. It is formulated with aluminium hydroxide as adjuvant. This vaccine comprises an attenuated strain of the HM-175 Hepatitis A virus inactivated with formol  
25 (formaldehyde); see Andre et. al. (Prog. med. Virol., vol. 37, p1-24).

As used herein, the term hepatitis A viral (HAV) antigen is used to refer to either a protein derived from hepatitis A virus or an attenuated strain of HAV, optionally inactivated, e.g. with formaldehyde. If the HAV antigen is a protein derived from  
30 hepatitis A virus it may optionally be a recombinant protein.

The vaccine Twinrix (Trade Mark) is a combination of a recombinant hepatitis B antigen with the aforementioned inactivated attenuated hepatitis A virus. The vaccine may be used to protect against hepatitis A and hepatitis B simultaneously.

- 5 European patent 0 339 667 (Chemo Sero) describes the general concept of combining a hepatitis A antigen and a hepatitis B antigen to make a combination vaccine. In that specification it is stated that the adjuvant which is used is not critical: it must only be capable of enhancing the immune activity to a desired extent and not cause any side-effects. It is stated that aluminium gel may be used, in  
10 particular aluminium hydroxide gel and aluminium phosphate gel.

In a further aspect, the invention provides a vaccine composition comprising:

- (a) a hepatitis B viral (HBV) antigen;
  - 15 (b) a herpes simplex viral (HSV) antigen; and
  - (c) an hepatitis A viral (HAV) antigen
- in combination with an adjuvant which is a preferential stimulator of TH1 cell response.
- 20 Such a vaccine is of great benefit for administration to adolescents who may be particularly at risk of HBV, and/or HSV infection, and/or HAV infection.

- An immune response may be broadly distinguished into two extreme categories, being a humoral or cell mediated immune responses (traditionally characterised by  
25 antibody and cellular effector mechanisms of protection respectively). These categories of response have been termed TH1-type responses (cell-mediated response), and TH2-type immune responses (humoral response).

- Extreme TH1-type immune responses may be characterised by the generation of  
30 antigen specific, haplotype restricted cytotoxic T lymphocytes, and natural killer cell responses. In mice TH1-type responses are often characterised by the generation of antibodies of the IgG2a subtype, whilst in the human these correspond

to IgG1 type antibodies. TH2-type immune responses are characterised by the generation of a broad range of immunoglobulin isotypes including in mice IgG1, IgA, and IgM.

- 5 It can be considered that the driving force behind the development of these two types of immune responses are cytokines. High levels of TH1-type cytokines tend to favour the induction of cell mediated immune responses to the given antigen, whilst high levels of TH2-type cytokines tend to favour the induction of humoral immune responses to the antigen.

10

- The distinction of TH1 and TH2-type immune responses is not absolute. In reality an individual will support an immune response which is described as being predominantly TH1 or predominantly TH2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4 +ve T cell clones by Mosmann and Coffman (*Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology, 7, p145-173*). Traditionally, TH1-type responses are associated with the production of the INF- $\gamma$  and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of TH1-type immune responses are not produced by T-cells, such as IL-12. In contrast, TH2- type responses are associated with the secretion of IL-4, IL-5, IL-6, IL-10 and tumour necrosis factor- $\beta$ (TNF- $\beta$ ).
- 15  
20  
25

- It is known that certain vaccine adjuvants are particularly suited to the stimulation of either TH1 or TH2 - type cytokine responses. Traditionally the best indicators of the TH1:TH2 balance of the immune response after a vaccination or infection includes direct measurement of the production of TH1 or TH2 cytokines by T lymphocytes *in vitro* after restimulation with antigen, and/or the measurement of the IgG1:IgG2a ratio of antigen specific antibody responses.
- 30

Thus, a TH1-type adjuvant is one which stimulates isolated T-cell populations to produce high levels of TH1-type cytokines when re-stimulated with antigen *in vitro*,

and induces antigen specific immunoglobulin responses associated with TH1-type isotype.

Adjuvants which are capable of preferential stimulation of the TH1 cell response are  
5 described in International Patent Application No. WO 94/00153 and WO 95/17209.

3 De-O-acylated monophosphoryl lipid A (3D-MPL) is one such adjuvant. This is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi  
10 Immunochem, Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in European Patent 0 689 454 B1 (SmithKline Beecham Biologicals SA).

Preferably, the particles of 3D-MPL are small enough to be sterile filtered through  
15 a 0.22micron membrane (as described in European Patent number 0 689 454). 3D-MPL will be present in the range of 10µg - 100µg preferably 25-50µg per dose wherein the antigen will typically be present in a range 2-50µg per dose.

Another preferred adjuvant comprises QS21, an Hplc purified non-toxic fraction  
20 derived from the bark of Quillaja Saponaria Molina. Optionally this may be admixed with 3 De-O-acylated monophosphoryl lipid A (3D-MPL), optionally together with an carrier.

The method of production of QS21 is disclosed in US patent No. 5,057,540.  
25

Non-reactogenic adjuvant formulations containing QS21 have been described previously (WO 96/33739). Such formulations comprising QS21 and cholesterol have been shown to be successful TH1 stimulating adjuvants when formulated together with an antigen. Thus vaccine compositions which form part of the present  
30 invention may include a combination of QS21 and cholesterol.

Further adjuvants which are preferential stimulators of TH1 cell response include immunomodulatory oligonucleotides, for example unmethylated CpG sequences as disclosed in WO 96/02555.

- 5 Combinations of different TH1 stimulating adjuvants, such as those mentioned hereinabove, are also contemplated as providing an adjuvant which is a preferential stimulator of TH1 cell response. For example, QS21 can be formulated together with 3D-MPL. The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1; preferably 1:5 to 5 : 1 and often substantially 1 : 1. The preferred  
10 range for optimal synergy is 2.5 : 1 to 1 : 1 3D-MPL: QS21.

Preferably a carrier is also present in the vaccine composition according to the invention. The carrier may be an oil in water emulsion, or an aluminium salt, such as aluminium phosphate or aluminium hydroxide.

15

A preferred oil-in-water emulsion comprises a metabolisable oil, such as squalene, alpha tocopherol and Tween 80. Additionally the oil in water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

- 20 In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with 3D-MPL and alum.

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1µg - 200µg, such as 10-100µg, preferably 10µg - 50µg per dose.

- 25 Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha tocopherol is equal to or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a  
30 stabiliser.



Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween 80, in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

- 5 A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210.

The HSV antigen in the composition of the invention is preferably derived from HSV-2, typically glycoprotein D. Glycoprotein D is located on the viral membrane,  
10 and is also found in the cytoplasm of infected cells (Eisenberg R.J. et al; J of Virol 1980, 35, 428-435). It comprises 393 amino acids including a signal peptide and has a molecular weight of approximately 60 kD. Of all the HSV envelope glycoproteins this is probably the best characterised (Cohen et al; J. of Virology, 60, 157-166). In vivo it is known to play a central role in viral attachment to cell  
15 membranes. Moreover, glycoprotein D has been shown to be able to elicit neutralising antibodies in vivo (Eing et al J. Med. Virology 127: 59-65). However, latent HSV-2 virus can still be reactivated and induce recurrence of the disease despite the presence of high neutralising antibodies titre in the patients sera.

- 20 An embodiment of the invention is a truncated HSV-2 glycoprotein D of 308 amino acids which comprises amino acids 1 through 306 naturally occurring glycoprotein with the addition Asparagine and Glutamine at the C terminal end of the truncated protein devoid of its membrane anchor region. This form of the protein includes the signal peptide which is cleaved to yield a mature 283 amino acid protein. The  
25 production of such a protein in Chinese Hamster ovary cells has been described in Genentech's European patent EP-B-139 417.

The recombinant mature HSV-2 glycoprotein D truncate is preferably used in the vaccine formulations of the present invention and is designated rgD2t.

30

A combination of this antigen in combination with the adjuvant 3D-MPL has been described in WO 92/16231.

The hepatitis B viral (HBV) antigen in the composition of the invention is typically hepatitis B surface antigen.

- 5 The preparation of Hepatitis B surface antigen (HBsAg) is well documented. See for example, Harford et. al. in Develop. Biol. Standard 54, page 125 (1983), Gregg et. al. in Biotechnology, 5, page 479 (1987), EP-A- 0 226 846, EP-A-0 299 108 and references therein.
- 10 As used herein the expression 'Hepatitis B surface antigen', abbreviated herein to 'HBsAg' or 'HBS' includes any HBsAg antigen or fragment thereof displaying the antigenicity of HBV surface antigen. It will be understood that in addition to the 226 amino acid sequence of the HBsAg S antigen (see Tiollais et. al. Nature, 317, 489 (1985) and references therein) HBsAg as herein described may, if desired,
- 15 contain all or part of a pre-S sequence as described in the above references and in EP-A- 0 278 940. HBsAg as herein described can also refer to variants, for example the 'escape mutant' described in WO 91/14703. In a further aspect the HBsAg may comprise a protein described as L\* in European Patent Application Number 0 414 374, that is to say a protein, the amino acid sequence of which
- 20 consists of parts of the amino acid sequence of the hepatitis B virus large (L) protein (ad or ay subtype), characterised in that the amino acid sequence of the protein consists of either:
- (a) residues 12 - 52, followed by residues 133 - 145, followed by residues 175 - 400 of the said L protein; or
- 25 (b) residue 12, followed by residues 14 - 52, followed by residues 133 - 145, followed by residues 175 - 400 of the said L protein.
- HBsAg may also refer to polypeptides described in EP 0 198 474 or EP 0 304 578.
- Normally the HBsAg will be in particle form. It may comprise S protein
- 30 alone or may be as composite particles, for example (L\*,S) wherein L\* is as defined above and S denotes the S-protein of hepatitis B surface antigen.

The HBsAg may be adsorbed on aluminium phosphate as described in WO93/24148.

Preferably the hepatitis B (HBV) antigen used in the formulation of the invention is  
5 HBsAg S-antigen as used in the commercial product Engerix-B (Trade Mark;  
SmithKline Beecham Biologicals).

A vaccine comprising hepatitis B surface antigen in conjunction with 3D-MPL was  
described in European Patent Application 0 633 784.

10

Epstein Barr Virus (EBV), a member of the herpesvirus group, causes infectious  
mononucleosis as a primary disease in humans. Predominantly it affects children  
or young adults. More than 90% of the average adult population is infected by  
EBV that persists for lifetime in peripheral B-lymphocytes. The virus is lifelong  
15 produced in the parotid gland and spread primarily by exchange of saliva from  
individuals who shed the virus. Children infected with EBV are largely  
asymptomatic or have very mild symptoms, while adolescents and adults who  
become infected develop typical infectious mononucleosis, characterised by fever,  
pharyngitis, and adenopathy. People who have been infected maintain anti-EBV  
20 antibodies for the remainder of their lives, and are thus immune to further infection.

In addition to its infectious qualities, EBV has been shown to transform  
lymphocytes into rapidly dividing cells and has therefore been implicated in several  
different lymphomas, including African Burkitt's lymphoma (BL). EBV may also  
25 be involved in causing nasopharyngeal carcinoma (NPC). Worldwide it is  
estimated that 80,000 cases of nasopharyngeal carcinoma occur and it is more  
prevalent in ethnic Chinese populations. Infectious mononucleosis is a consequence  
of primary infection by EBV. It is not a life-threatening disease if additional risk  
factors are absent.

30

Four proteins of the EBV viral envelope constituting the so-called membrane  
antigen complex have been described. They are usually referred to as gp 220/350

or gp 250/350 or simply as gp 250 or 350 (see EP-A-151079). There is convincing evidence that gp 350 and gp 250 induce the production of neutralising antibodies and that antibodies against gp 350 and gp 250 have neutralising capacity. These proteins are thus candidates for a possible EBV vaccine. For further information  
5 about the application of gp 250/350 for prophylaxis and treatment of EBV-related diseases see EP 0 173 254.

The major EBV surface glycoprotein gp350/220 infects human target cells through interaction with the cellular membrane protein , CD21. Gp350/220 is the primary  
10 target for EBV-neutralising antibodies in humans and some forms of gp350/220 have been shown to protect against EBV-related disease. Preferably a vaccine composition according to the invention comprises gp 350 of EBV although other protective antigens may be used.

15 Papillomaviruses are small DNA tumour viruses, which are highly species specific. As yet, over 70 individual human papillomavirus (HPV) genotypes have been described. HPVs are generally specific either for the skin (e.g. HPV-1 and -2) or mucosal surfaces (e.g. HPV-6 and -11) and usually cause benign tumours (warts) that persist for several months or years. Such benign tumours may be distressing  
20 for the individuals concerned but tend not to be life threatening, with a few exceptions.

Some HPVs are also associated with cancers. The strongest positive association between an HPV and human cancer is that which exists between HPV-16 and HPV-  
25 18 and cervical carcinoma. Cervical cancer is the most common malignancy in developing countries, with about 500,000 new cases occurring in the world each year. It is now technically feasible to actively combat primary HPV-16 infections, and even established HPV-16-containing cancers, using vaccines. For a review on the prospects for prophylactic and therapeutic vaccination against HPV-16 see  
30 Cason J., Clin. Immunother. 1994; 1(4) 293-306 and Hagenessee M.E., Infections in Medicine 1997 14(7) 555-556,559-564. Preferably a vaccine composition according to the invention comprises the major capsid protein, the L1 protein.

Today, the different types of HPVs have been isolated and characterised with the help of cloning systems in bacteria and more recently by PCR amplification. The molecular organisation of the HPV genomes has been defined on a comparative basis with that of the well characterised bovine papillomavirus type 1 (BPV1).

Although minor variations do occur, all HPVs genomes described have at least seven early genes, E1 to E7 and two late genes L1 and L2. In addition, an upstream regulatory region harbors the regulatory sequences which appears to control most transcriptional events of the HPV genome.

E1 and E2 genes are involved in viral replication and transcriptional control, respectively and tend to be disrupted by viral integration. E6 and E7, and recent evidence implicate also E5 are involved in viral transformation.

In the HPVs involved in cervical carcinoma such as HPV 16 and 18, the oncogenic process starts after integration of viral DNA. The integration results in the inactivation of genes coding for the capsid proteins L1 and L2 and in installing continuously over expression of the two early proteins E6 and E7 that will lead to gradually loss of the normal cellular differentiation and the development of the carcinoma.

Carcinoma of the cervix is common in women and develops through a pre-cancerous intermediate stage to the invasive carcinoma which frequently leads to death. The intermediate stages of the disease is known as cervical intraepithelial neoplasia and is graded I to III in terms of increasing severity.

Clinically, HPV infection of the female anogenital tract manifests as cervical flat condylomas, the hallmark of which is the koilocytosis affecting predominantly the superficial and intermediate cells of the cervical squamous epithelium.

30

Koilocytes which are the consequence of a cytopathic effect of the virus, appear as multinucleated cells with a perinuclear clear halo. The epithelium is thickened with abnormal keratinisation responsible for the warty appearance of the lesion.

- 5 Such flat condylomas when positive for the HPV 16 or 18 serotypes, are high-risk factors for the evolution toward cervical intraepithelial neoplasia (CIN) and carcinoma in situ (CIS) which are themselves regarded as precursor lesions of invasive cervix carcinoma.
- 10 International Patent Application No. WO 96/19496 discloses variants of human papilloma virus E6 and E7 proteins, particularly fusion proteins of E6/E7 with a deletion in both the E6 and E7 proteins. These deletion fusion proteins are said to be immunogenic.
- 15 HPV L1 based vaccines are disclosed in WO94/00152, WO94/20137, WO93/02184 and WO94/05792. Such a vaccine can comprise the L1 antigen as a monomer, a capsomer or a virus like particle. Such particles may additionally comprise L2 proteins. Other HPV vaccines are based on the Early proteins, such as E7 or fusion proteins such as L2-E7.
- 20 In the vaccine of the invention it is preferred to utilise compositions comprising either an E6 or E7 protein linked to an immunological fusion partner having T cell epitopes.
- 25 In a preferred form of the invention, the immunological fusion partner is derived from protein D of Haemophilus influenza B. Preferably the protein D derivative comprises approximately the first 1/3 of the protein, in particular approximately the first N-terminal 100-110 amino acids.
- 30 Accordingly the present invention in one embodiment comprises antigen (s) derived from HPV as described above. Preferably the invention comprises fusion proteins comprising Protein D - E6 from HPV 16, Protein D - E7 from HPV 16 Protein D -

E7 from HPV 18 and Protein D - E6 from HPV 18. The protein D part preferably comprises the first 1/3 of protein D.

The proteins of the present invention preferably are expressed in *E. coli*. In a preferred embodiment the proteins are expressed with a Histidine tail comprising between 5 to 9 and preferably six Histidine residues. These are advantageous in aiding purification. The description of the manufacture of such proteins is fully described in co-pending UK patent application number GB 9717953.5.

- 10 In a preferred aspect the vaccine composition of the invention additionally comprises a Varicella Zoster viral antigen (VZV antigen). Suitable antigens of VZV for inclusion in the vaccine formulation include gpI-V described by Longnecker et al., Proc Natl Acad Sci USA 84, 4303-4307 (1987).
- 15 In a preferred embodiment gpI (see Ellis et al., US patent 4,769,239) is used. See also European Patent No. 0 405 867 B1.

In another preferred aspect the vaccine composition of the invention additionally comprises a human cytomegalovirus (HCMV) antigen. HCMV is a human DNA virus belonging to the family of herpes viruses. HCMV is endemic in most parts of the world. Among two populations, HCMV is responsible for serious medical conditions. HCMV is a major cause of congenital defects in new borns. The second population at risk are immunocompromised patients such as those suffering from HIV infection and those patients undergoing transplantations. The clinical disease causes a variety of symptoms including fever, hepatitis, pneumonitis and infectious mononucleosis. A preferred antigen for use in a vaccine against HCMV is gB685\*\* as described in WO 95/31555. Immunogens for use in HCMV vaccines are also provided by pp65, an HCMV Matrix Protein as described in WO 94/00150 (City of Hope).

In one preferred aspect the vaccine composition of the invention additionally comprises both a VZV and an HCMV antigen, in particular those antigens described above.

- 5 In another preferred aspect the vaccine composition of the invention additionally comprises a *Toxoplasma gondii* antigen. *Toxoplasma gondii* is an obligate intracellular protozoan parasite responsible for toxoplasmosis in warm-blooded animals, including man. Although it is generally clinically asymptomatic in healthy individuals, toxoplasmosis may cause severe complications in pregnant women and  
10 immunocompromised patients. A preferred antigen for use in a vaccine against toxoplasma gondii is SAG1 ( also known as P30) as described in WO96/02654 or Tg34 as described in WO92/11366.

- In one preferred aspect the vaccine composition of the invention additionally  
15 comprises either a VZV antigen or an HCMV antigen combined with a *Toxoplasma gondii* antigen, in particular those antigens described above.

- In a preferred aspect the vaccine composition of the invention is a multivalent vaccine, for example a tetra- or pentavalent vaccine.  
20

The formulations of the present invention are very effective in inducing protective immunity, even with very low doses of antigen (e.g. as low as 5µg rgD2t).

- They provide excellent protection against primary infection and stimulate,  
25 advantageously both specific humoral (neutralising antibodies) and also effector cell mediated (DTH) immune responses.

- The present invention in a further aspect provides a vaccine formulation as herein described for use in medical therapy, particularly for use in the treatment or  
30 prophylaxis of Herpes Simplex infections and hepatitis B viral infections.



The vaccine of the present invention will contain an immunoprotective quantity of the antigens and may be prepared by conventional techniques.

Vaccine preparation is generally described in Pharmaceutical Biotechnology, Vol.61 Vaccine Design - the subunit and adjuvant approach, edited by Powell and Newman, Plenum Press, 1995. New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for example, by Likhite, U.S. Patent 4,372,945 and by Armor et al., U.S. Patent 4,474,757.

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed. Generally, it is expected that each dose will comprise 1-1000 $\mu$ g of protein, preferably 2-100 $\mu$ g, most preferably 4-40 $\mu$ g. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of antibody titres and other responses in subjects. Following an initial vaccination, subjects may receive a boost in about 4 weeks.

In addition to vaccination of persons susceptible to HSV or HBV viral infections, the pharmaceutical compositions of the present invention may be used to treat, immunotherapeutically, patients suffering from the said viral infections.

In a further aspect of the present invention there is provided a method of manufacture as herein described, wherein the method comprises mixing a herpes viral antigen and a hepatitis B viral antigen with a TH-1 inducing adjuvant, for example 3D-MPL and, preferably, a carrier, for example alum.

If desired, other antigens may be added, in any convenient order, to provide multivalent vaccine compositions as described herein.

**Example 1: Immunogenicity study with gD + HBs combination**

The objective of the study was to demonstrate the feasibility of the HSV  
5 gD/HBV HBs combination in Al(OH)<sub>3</sub> / 3D-MPL formulation. Immune responses  
induced in guinea pigs by immunisation with these antigens used alone or in  
combination were compared. HBs is an abbreviation for Hepatitis B surface  
antigen, specifically the S-protein as described hereinabove. gD is an abbreviation  
for rgD<sub>2</sub>t as described hereinabove.

10

**Experimental protocol**

The experimental protocol was the following. Groups of 6 female Hartley  
guinea pigs were injected intramuscularly on day 0 and day 28 with the following  
15 formulations:

- group 1: HBs 5 µg / Al(OH)<sub>3</sub> 125 µg / 3D-MPL 12.5 µg
- group 2: gD 5 µg / Al(OH)<sub>3</sub> 125 µg / 3D-MPL 12.5 µg
- group 3: HBs 5 µg + gD 5 µg / Al(OH)<sub>3</sub> 125 µg / 3D-MPL 12.5 µg
- 20 - group 4: HBs 5 µg + gD 5 µg / Al(OH)<sub>3</sub> 125 µg / 3D-MPL\* 12.5 µg

\* different 3D-MPL batch

Animals were bled 14 and 31 days after the second immunization. The  
25 humoral immune response against HSV gD and HBV HBs was evaluated at both  
time points in ELISA.

Delayed type hypersensitivity (DTH) reactions were also evaluated for HBs.  
They consisted in the intradermal injection of 10 µg HBs in duplicate. The  
30 development of DTH reactions was monitored by measuring skin thickness at 0, 24  
and 48 hours after injection.

## Results

### 1. Antibody responses

5

Anti-gD ELISA titers are shown in Fig. 1. Anti-gD titers in the group immunized with gD were comparable to those induced in animals immunized with the gD + HBs combination. The presence of HBs in the formulation did not affect the induction of anti-gD antibody responses.

10

Similarly, anti-HBs antibody titers were compared in animals immunized with HBs alone or combined with gD. Fig. 2 shows that comparable anti-HBs titers were observed in animals immunized with HBs alone or with HBs + gD.

15

The results are shown in Figures 1-4 from which it may be concluded that in the tested formulation, the gD / HBs combination induces antibody responses comparable to those induced by the same antigens used alone and DTH responses to HBs comparable to those induced by HBs alone. Thus no significant differences in DTH responses to HBs are observed in HBs or HBs + gD vaccinated animals. The presence of gD did not affect the DTH response to HBs.

20

### Example 2: PRO30 Experiment HBV/HSV combination

25

The objective of this study was to evaluate in the HSV guinea pig model the protective efficacy of an HSV gD + HBV HBs combination in a 3D-MPL/alum formulation as compared to gD alone in a 3D-MPL/alum formulation. The 3D-MPL/alum formulation comprises alum (10 parts by weight) to 3D-MPL (1 part by weight).

30

## Experimental Protocol

Groups of twelve females Hartley guinea pigs were immunized with the following formulations or left untreated:

5

gD + HBs / 3D-MPL/alum formulation	gD (5 $\mu$ g)	Al(OH) <sub>3</sub> , (62.5 $\mu$ g)	3D-MPL (6.25 $\mu$ g)
	+ HBs (5 $\mu$ g)	+ Al(OH) <sub>3</sub> , (62.5 $\mu$ g)	+ 3D-MPL (6.25 $\mu$ g)

gD / 3D-MPL/alum formulation	gD (5 $\mu$ g)	Al(OH) <sub>3</sub> , (125 $\mu$ g)	3D-MPL (12.5 $\mu$ g)
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Animals were immunized intramuscularly twice at days 0 and 28. They were intravaginally challenged at day 57 (1 month after the second immunization) with 10<sup>5</sup> pfu HSV2 MS strain (100 $\mu$ l) and then daily monitored for clinical signs of primary (days 4 to 12 post-infection) and recurrent (days 13 to 39 post-infection) disease. The protection induced was measured according to several criteria described in Table 1, as well as by cumulative score curves.

Sera collected at days 14 and 28 post II immunization were also tested for their anti-gD ELISA Ab titers (expressed as EU/ml); sera obtained at day 28 post II were also tested for their HSV neutralizing activity ('NEUTRA'; titers correspond to the reciprocal of the serum dilution giving 100% protection against HSV2 cytopathic effect).

## Results

### Serology results:

Immunogenicity data are presented in the following Table and Figure 5:

Neutra / ELISA ratio at days 28 post II			
FORMULATIONS	ELISA (GMT)	NEUTRA (GMT)	Ratio NEUTRA / ELISA
gD + HBs / 3D-MPL/alum formulation	6687	238	3.5 %
gD / 3D-MPL/alum formulation	7475	200	2.7 %

Similar ELISA and neutralizing titers were induced by the gD (5 $\mu$ g) + HBs (5 $\mu$ g) combination in 3D-MPL/alum formulation and gD / 3D-MPL/alum formulation.

**Protection against primary disease:**

As shown in Figure 6 (cumulative score curve) and in Table 1 below, gD + HBs / 3D-MPL/alum formulation combination conferred as good protection against primary disease as gD alone in 3D-MPL/alum formulation.

**Protection against recurrent disease:**

Protection against herpes recurrences is shown in Figure 7 (cumulative score curves) and in Table 2 below. gD + HBs / 3D-MPL/alum formulation combination conferred as good protection against recurrent disease as gD alone in 3D-MPL/alum formulation.

Similar number of animals had recurrences in the gD + HBs and gD alone groups. Exactly the same number of animals in these groups had more than 1 recurrence during the observation period (day 13 to 39 post challenge). In those animals with recurrences, comparable lesion severity were recorded.

Table 1: Primary Disease

GRO UP	n	FORMULA TION	PRIMARY DISEASE					Lesion severity *		
			Animal without lesion	Vaginal Lesions Incidence %	External lesions Incidence %	PI index**		medi an	% vs control	n
1	1	gD/3D-	75.0	16.7	8.3	25.0	-	0.50	-96%	3
	2	MPL/alum				96%				
2	1	gD +	83.3	0.0	16.7	50.0	-	9.00	-22%	2
	2	HBs/3D- MPL/alum				91%				
3	1	Untreated	16.7	8.3	75.0	587.5		11.5		10
	2							0		

\* Sum of the lesion scores for the days 4 to 12 post-infection (animals without lesion are considered).

Lesion scores: no lesion (0), vaginal lesions (0.5 or 1), external skin vesicles (2, 4, 8 or 16)

\*\*Primary infection index = sum (Max. score i) x (Incidence %); with i=0, 0.5, 1, 2, 4, 8 or 16

Both guinea pigs immunized with the gD + HBs combination and guinea pigs immunized with gD alone were very well protected against HSV challenge. While most control animals had lesions, these were observed in respectively 2 / 12 and 3 / 12 animals only in the gD + HBs and gD alone groups.

Table 2: Recurrent Disease

n	FORMULATION	RECURRENT DISEASE									
		Animals w/o rec.			Animals with recurrence						
		With PI incidence %	Total %	w/o PI Incidence %	With P.I. Incidence %	Total lesion %	More than 1 recurrence %	Lesion Severity *			Duration
								Median	% vs control	n	Median
12	gD/3D-MPL/alum	8.3	58.3	25.0	16.7	41.7	16.6	3.00	-57%	5	4.00
12	gD + HBs/3D-MPL/alum	8.3	66.7	25.0	8.3	33.3	16.6	5.50	-21%	4	3.50
12	Untreated	8.3	25.0	0.0	75.0	75.0	50	7.00		9	6.00

**Example 3:**

The objective of the study is to evaluate the serological immune responses induced  
5 in mice by a combination vaccine comprising HAV, HBs and gD formulated with  
Aluminium salts and 3D-MPL. The hepatitis A component used in this example  
(and abbreviated herein to 'HAV') was the inactivated HM175 strain found in  
Havrix.

**10 Materials and Methods**

Antigen / 3D-MPL batches:

HBs: Al:4550  $\mu\text{g/ml}$ , pre-adsorbed HBs: 227,62  $\mu\text{g/ml}$   
15 HAV: Al:1380  $\mu\text{g/ml}$ , HAV:25230 EU/ml  
gD: 493  $\mu\text{g/ml}$   
3D-MPL: 957  $\mu\text{g/ml}$

**Formulation process**

- 20 • Group 1: HBs  $\text{AlPO}_4$  /3D-MPL  
HBs /  $\text{H}_2\text{O}$  / NaCl / phenoxy /  $\text{AlPO}_4$  for 15 min + 3D-MPL for 1 hr
- Group 2: gD  $\text{AlOH}_3$ /3D-MPL  
 $\text{H}_2\text{O}$  /  $\text{AlOH}_3$  for 5 min + gD for 15 min + 3D-MPL for 30 min + PBS for 15  
min + Phenoxy
- 25 • Group3: HAV  $\text{AlOH}_3$ /3D-MPL  
 $\text{H}_2\text{O}$  /NaCl / Phenoxy for 5 min +  $\text{AlOH}_3$ /HAV for 30 min / 3D-MPL
- Group 4:  
1.  $\text{H}_2\text{O}$  / NaCl / Phenoxy for 5 min +  $\text{AlPO}_4$  / HBs for 5 min + 3D-MPL 30 min  
2. gD /  $\text{AlOH}_3$  for 15 min + 3D-MPL for 30 min
- 30 Mix 1 and 2 for 20min + HAV for 1hr.



### Serological read-outs

5

- Quantitation of anti-HBs and anti-gD antibody was performed by Elisa using HBs or gD as coating antigen. Antigen and antibody solutions were used at 50  $\mu$ l per well. Antigen was diluted at a final concentration of 1  $\mu$ g/ml in PBS and was adsorbed overnight at 4°C to the wells of 96 wells microtiter plates (Maxisorb Immuno-plate, Nunc, Denmark). The plates were then incubated for 1hr at 37°C with PBS containing 1% bovine serum albumin and 0.1% Tween 20 (saturation buffer). Two-fold dilutions of sera in the saturation buffer were added to the antigen-coated plates and incubated for 1 hr 30 min at 37°C. The plates were washed four times with PBS 0.1% Tween 20 and biotin-conjugated anti-mouse Ig (Amersham, UK) diluted 1/1000 in saturation buffer was added to each well and incubated for 1 hr 30 min at 37°C. After a washing step, streptavidin-biotinylated peroxylase complex (Amersham, UK) diluted 1/5000 or 1/1000 in saturation buffer (for HBs and anti-gD ELISA respectively) was added for an additional 30 min at 37°C. Plates were washed as above and incubated for 20 min with a solution of o-phenylenediamine (Sigma) 0.04% H<sub>2</sub>O<sub>2</sub> 0.03% in 0.1% tween 20 0.05M citrate buffer pH4.5. The reaction was stopped with H<sub>2</sub>SO<sub>4</sub> 2N and read at 492/620 nm. ELISA titers were calculated from a reference by SoftmaxPro (using a four parameters equation ) and expressed in EU/ml.
- Quantitation of anti-HAV antibody was performed by Enzymun ELISA (Boehringer) according to Manufacturer's protocol.

30

### **Experimental Protocol**

Groups of 7 Balb/C mice were immunised intramuscularly with the following formulations (corresponding to 1/10 human dose):

1. HBs (2  $\mu$ g) / AlPO<sub>4</sub> (50  $\mu$ g) / 3D-MPL (5  $\mu$ g)
- 5 2. gD (2 $\mu$ g) / Al(OH)<sub>3</sub> (50  $\mu$ g) / 3D-MPL (5  $\mu$ g)
3. HAV (72EU) / Al(OH)<sub>3</sub> (50  $\mu$ g) / 3D-MPL (5  $\mu$ g)
4. HAV (72U) / Al(OH)<sub>3</sub> (5 $\mu$ g) + HBs (2  $\mu$ g) / AlPO<sub>4</sub> (40 $\mu$ g) / 3D-MPL (2.5  $\mu$ g)  
+ gD (2 $\mu$ g) / Al(OH)<sub>3</sub> (5  $\mu$ g) / 3D-MPL(2.5  $\mu$ g)

10

Animals were immunised twice at day 0 and 21 with 50 $\mu$ l vaccine. Sera were collected at various time points post-immunisations (21 post I and 14 post II) and were tested for their anti-HAV, HBs and gD antibody titers .

## 15 Results

Individual data from 21 post I and 14 post II are shown in Table 3 and summarised below:

Table 3:

Anti HBs response (EU/ml)		Anti-HAV response (mIU/ml)		Anti-gD response (EU/ml)		
	14 post I	14 post II	14 post I	14 post II	14 post I	14 post II
<b>Gr.1 DHB56A2: HBs 20 / PO4 / 3D-MPL 50</b>						
	659	127282				
	935	165796				
	979	118574				
	934	55063				
	1606	102040				
	879	90388				
	409	39447				
<b>GMT</b>	<b>880</b>	<b>75015</b>				
<b>Gr.2 gD 20 / OH / 3D-MPL 50</b>						
					989	149616
					1150	97672
					564	61866
					1087	100172
					805	73340
					1135	186113
					598	122633

Table 3 cont.

GMT						871	106123
Gr.3	HAV 720 / OH / 3D-MPL						
	50						
				20	20		
				20	20		
				20	41		
				20	20		
				20	37		
				20	20		
				20	20		
	GMT			20	25		
Gr.4	HAV 720 / OH - HBs 20 / PO4 / 3D-MPL 25 - gD 20 /						
	OH / 3D-MPL 25						
	88	63427	25	272	202	61711	
	336	72765	20	28	1166	105676	
	395	78781	20	498	730	65277	
	474	99881	20	380	819	62107	
	890	112439	20	20	395	76378	
	542	120652	20	20	493	54908	
	1020	41460	20	20	345	35466	
	GMT	414	79699	21	91	549	69312

5 • HBs serology

Group	Anti-HBs ELISA titers (EU/ml)	
	Post I	Post II
HBs AlPO4 3D-MPL	880	75015
HAV Al(OH)3 / HBs AlPO4 3D-MPL / gD	414	79699
Al(OH)3 3D-MPL		

- gD serology

Group	Anti-gD ELISA titers (EU/ml)	
	Post I	Post II
gD Al(OH) <sub>3</sub> 3D-MPL	871	106123
HAV Al(OH) <sub>3</sub> / HBs AlPO <sub>4</sub> 3D-MPL / gD	549	69312
Al(OH) <sub>3</sub> 3D-MPL		

5 • HAV serology

Group	Anti-HAV ELISA titers (mIU/ml)	
	Post I	Post II
HAV Al(OH) <sub>3</sub> 3D-MPL	20	25
HAV Al(OH) <sub>3</sub> / HBs AlPO <sub>4</sub> 3D-MPL / gD	21	91
Al(OH) <sub>3</sub> 3D-MPL		

## 10 Conclusions

- Comparable anti-HBs antibody titers in the combination vaccine and in HBs vaccine containing Aluminium salts and 3D-MPL were observed.
- Comparable anti-gD antibody titers in the combination vaccine and in gD vaccine containing Aluminium salts and 3D-MPL were observed.
- Comparable anti-HAV antibody titers in the combination vaccine and in HAV vaccine containing Aluminium salts and 3D-MPL were observed.

Thus there appears to be no interference when HBs, gD and HAV are combined in a vaccine containing aluminium salts and 3D-MPL.

**Claims**

1. A vaccine composition comprising:
  - (a) a hepatitis B viral (HBV) antigen; and
  - 5 (b) a herpes simplex viral (HSV) antigenin conjunction with an adjuvant which is a preferential stimulator of TH1 cell response.
- 10 2. A vaccine composition according to claim 1 which additionally comprises a carrier.
3. A vaccine composition according to claim 1 or claim 2 in which the preferential stimulator of TH1-cell response is selected from the group of adjuvants comprising: 3D-MPL, 3D-MPL wherein the size of the particles of 3D-MPL is  
15 preferably about or less than 100nm, QS21, a mixture of QS21 and cholesterol, and a CpG oligonucleotide.
4. A vaccine composition according to claim 3 in which the preferential  
20 stimulator of TH1-cell response is 3D-MPL.
5. A vaccine composition according to any one of claims 1 to 4 in which the Hepatitis B antigen is hepatitis B surface antigen
6. A vaccine composition according to any one of claims 1 to 5 in which the  
25 HSV antigen is HSV2 gD or a truncate thereof.
7. A vaccine composition according to any one of claims 1 to 6 in which an EBV antigen is additionally present.
- 30 8. A vaccine composition as defined in claim 7 in which the EBV antigen is gp 350.

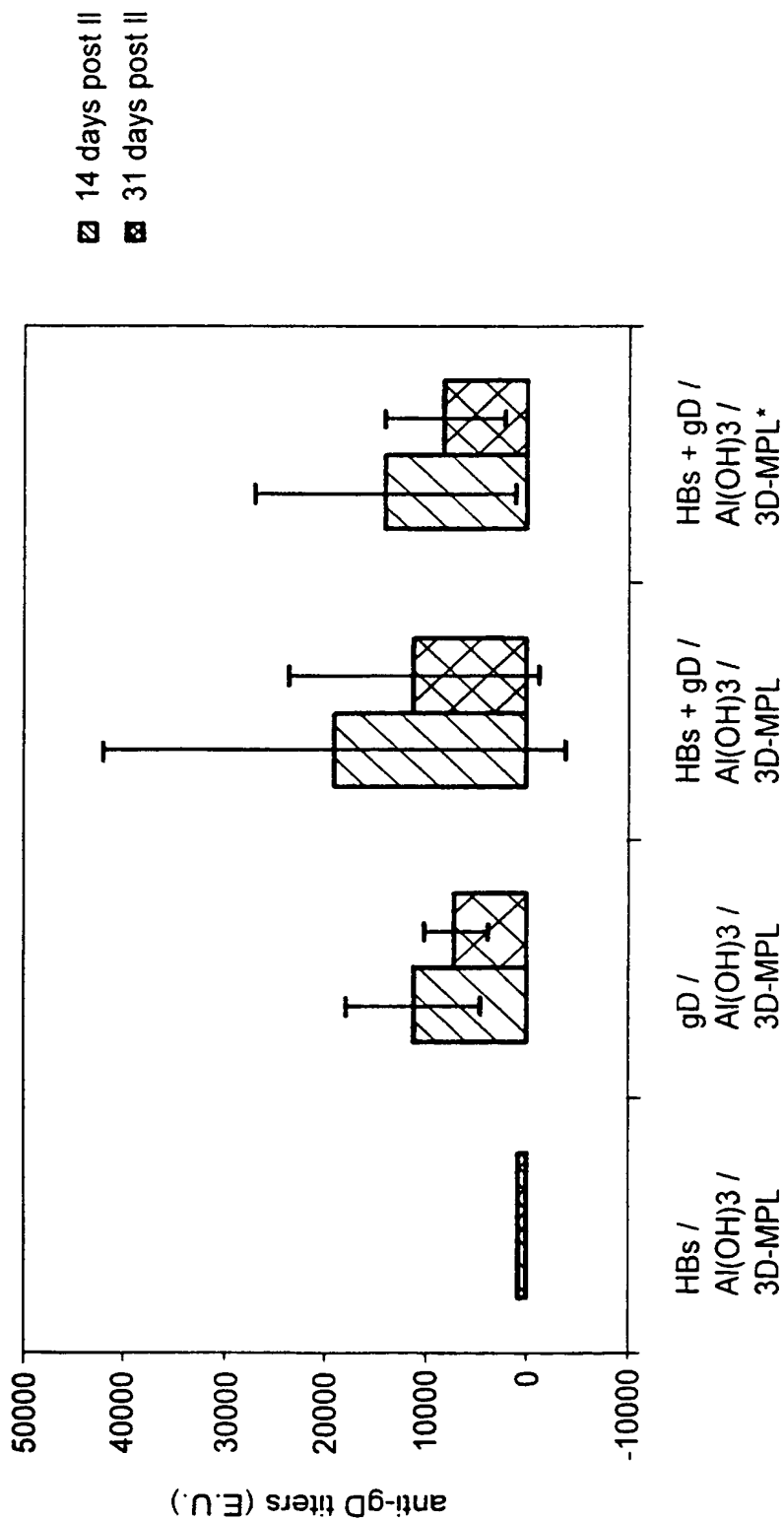
9. A vaccine composition according to any one of claims 1 to 6 in which a hepatitis A antigen is additionally present.
10. A vaccine composition as defined in claim 9 in which the HAV antigen is  
5 derived from the HM-175 strain.
11. A vaccine composition according to any one of claims 1 to 6 in which a human papilloma virus (HPV) antigen is additionally present.
- 10 12. A vaccine composition as defined in claim 11 in which the HPV antigen is selected from one or more of the following; L1, L2, E6, E7, protein D-E6, protein D-E7 or L2-E7.
13. A vaccine composition according to any one of claims 1 to 12 in which the  
15 carrier is selected from the group comprising aluminium hydroxide, aluminium phosphate and tocopherol and an oil in water emulsion.
14. A vaccine composition according to any one of claims 1 to 13 which additionally comprises a VZV antigen.
- 20 15. A vaccine composition according to claim 14 in which the VZV antigen is gpl.
16. A vaccine composition according to any one of claims 1 to 15 which  
25 additionally comprises a HCMV antigen.
17. A vaccine composition according to claim 16 in which the HCMV antigen is gB685\*\* or pp65.
- 30 18. A vaccine composition according to any one of claims 1 to 17 which additionally comprises a Toxoplasma gondii antigen.

19. A vaccine composition according to claim 18 in which the *Toxoplasma gondii* antigen is SAG1 or TG34.
20. A vaccine composition according to any one of claims 1 to 4 comprising
- 5 HBsAg S antigen and HSV2gDt and optionally in addition one or more of EBVgp 350; VZVgpI,; HAV HM-175 inactivated strain; L1, L2, E6, E7, protein D-E6, protein D-E7 or L2-E7 of HPV ; gB685\*\* or pp65 of HCMV and SAG1 or TG34 antigens of *Toxoplasma gondii*.



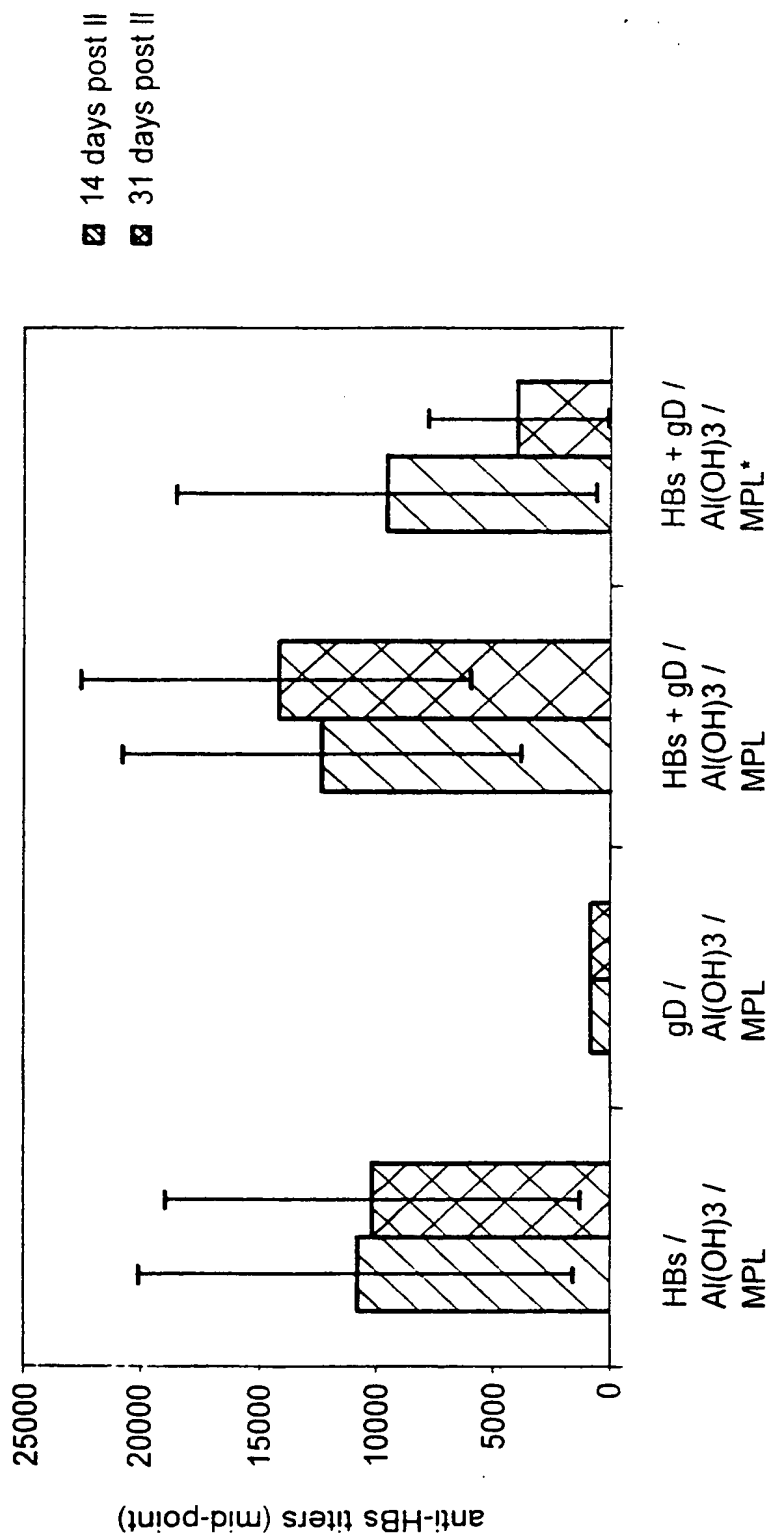
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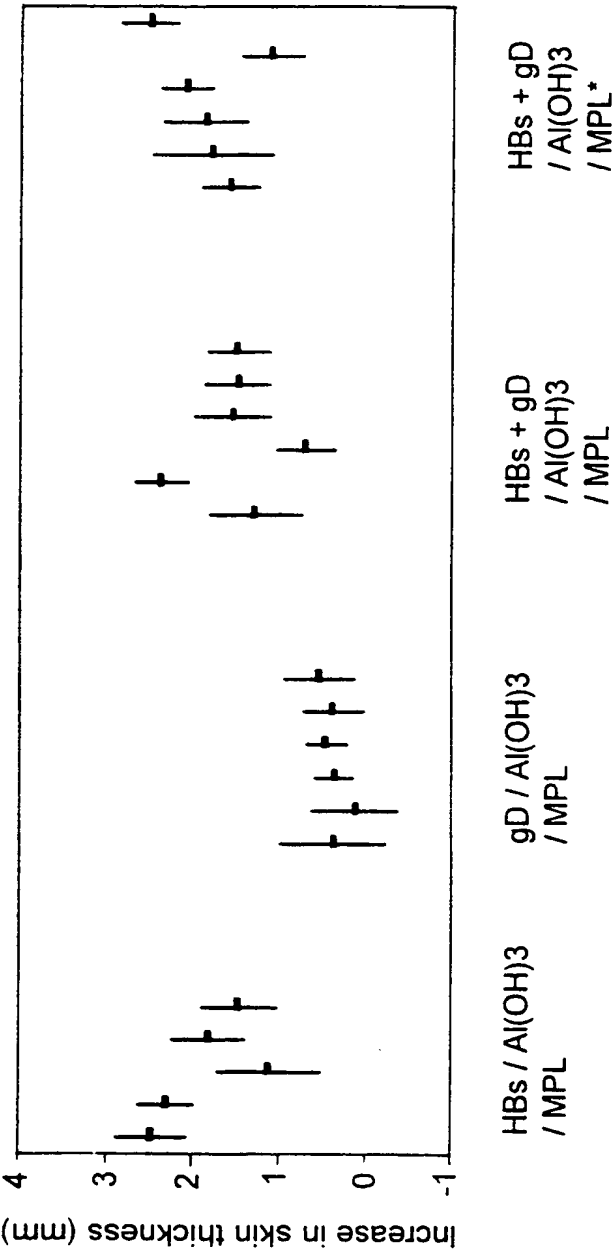


**Fig. 1** anti-gD ELISA titers

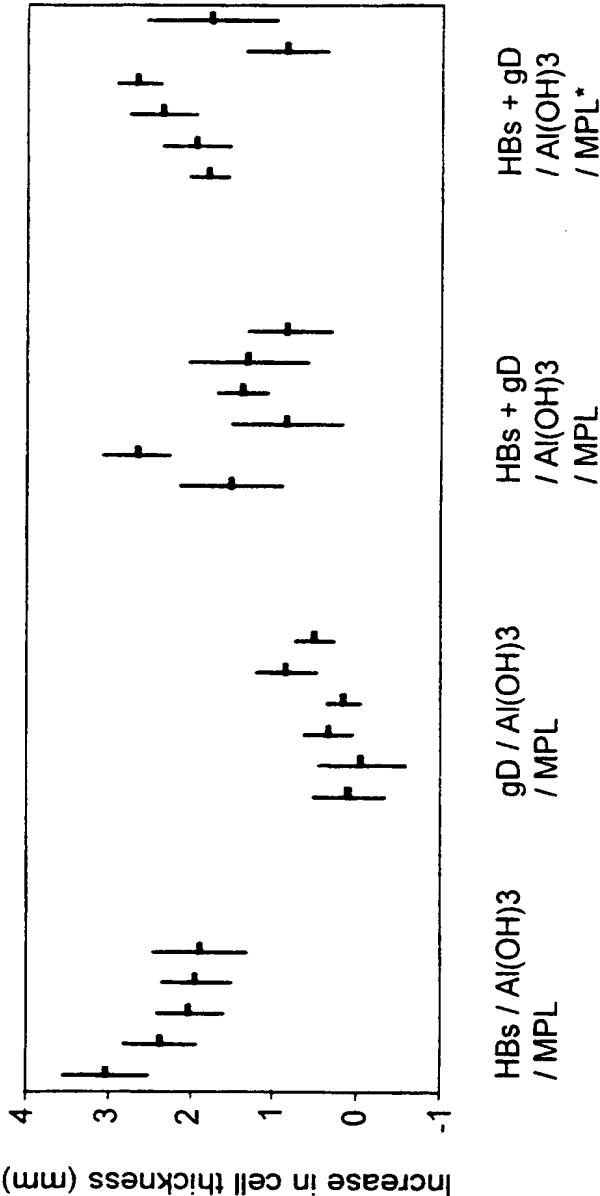
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**Fig. 2** anti-HBs ELISA titers

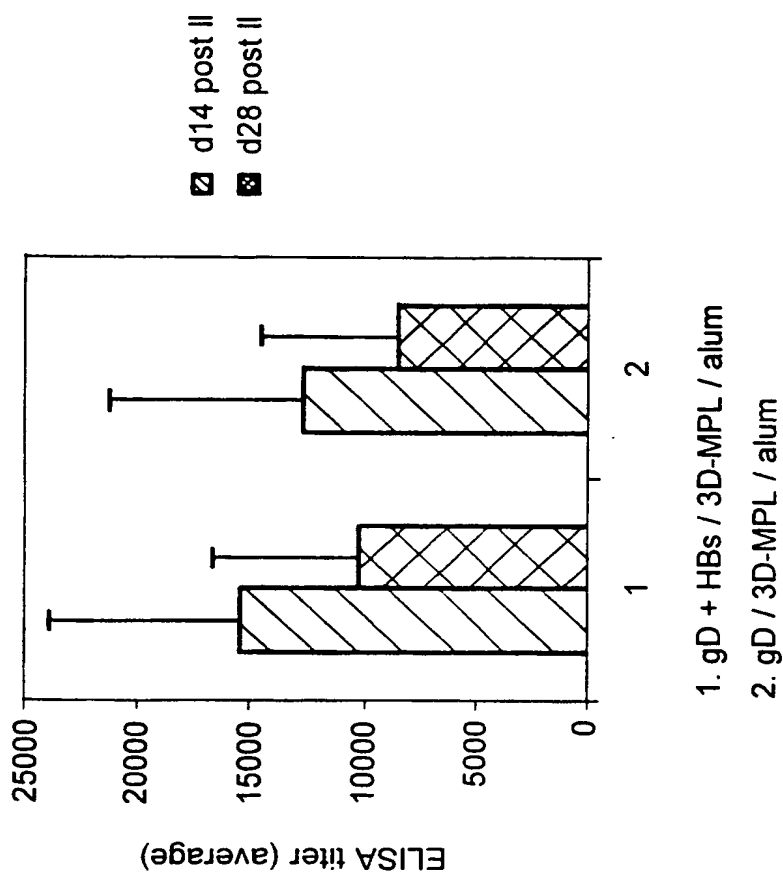


**Fig. 3** DTH reaction 24 hrs



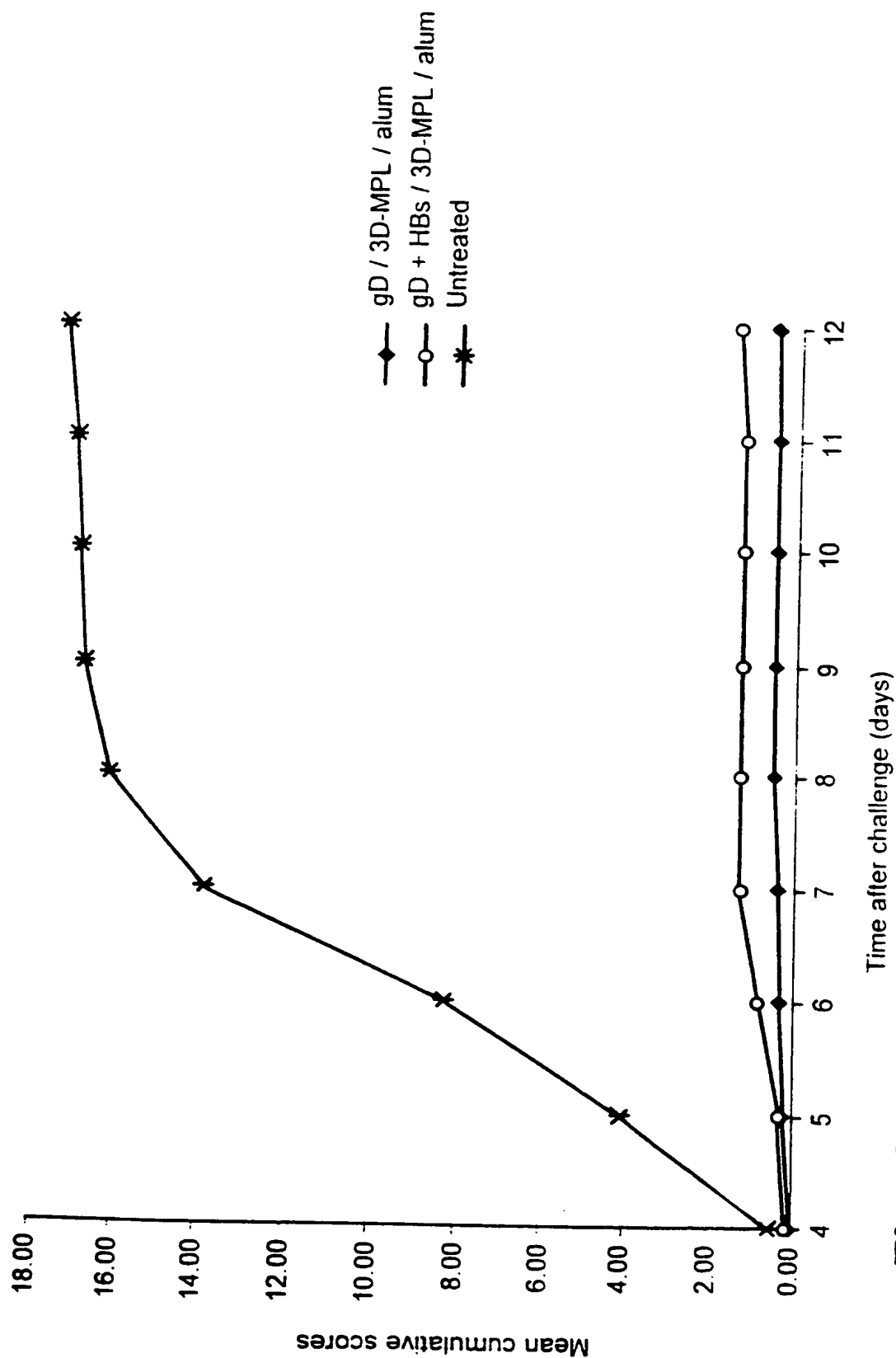
**Fig. 4** DTH reaction at 48 hrs

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**Fig. 5**

Similar ELISA and neutralizing titers were induced by the gD (5 $\mu$ g) + HBs (5 $\mu$ g) combination in 3D-MPL / alum and gD / 3D-MPL / alum.

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**Fig. 6**

Both guinea pigs immunized with the gD + HBs combination and guinea pigs immunized with gD alone were very well protected against HSV challenge. While most control animals had lesions, these were observed in respectively 2 / 12 and 3 / 12 animals only in the gD + HBs and gD alone groups.

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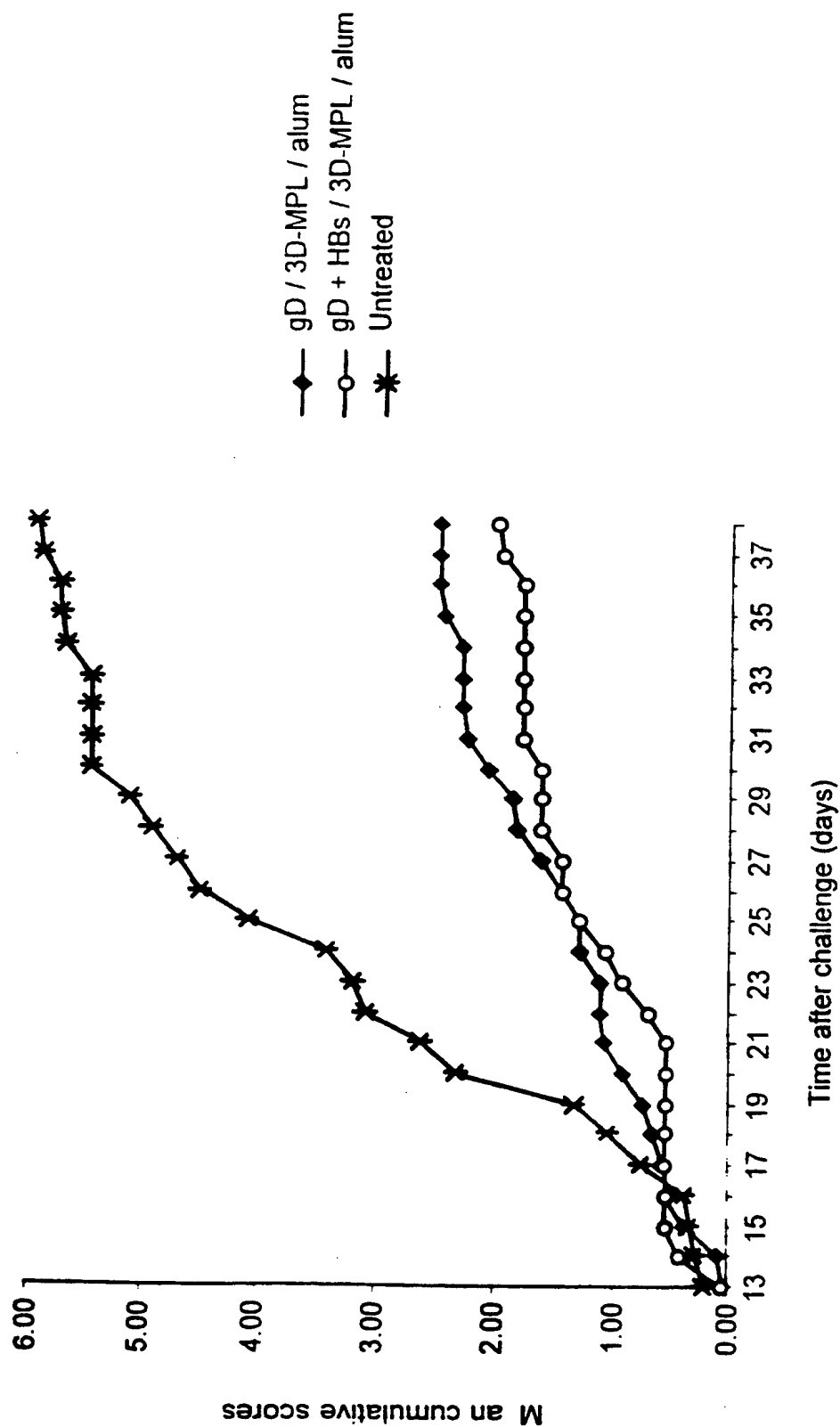
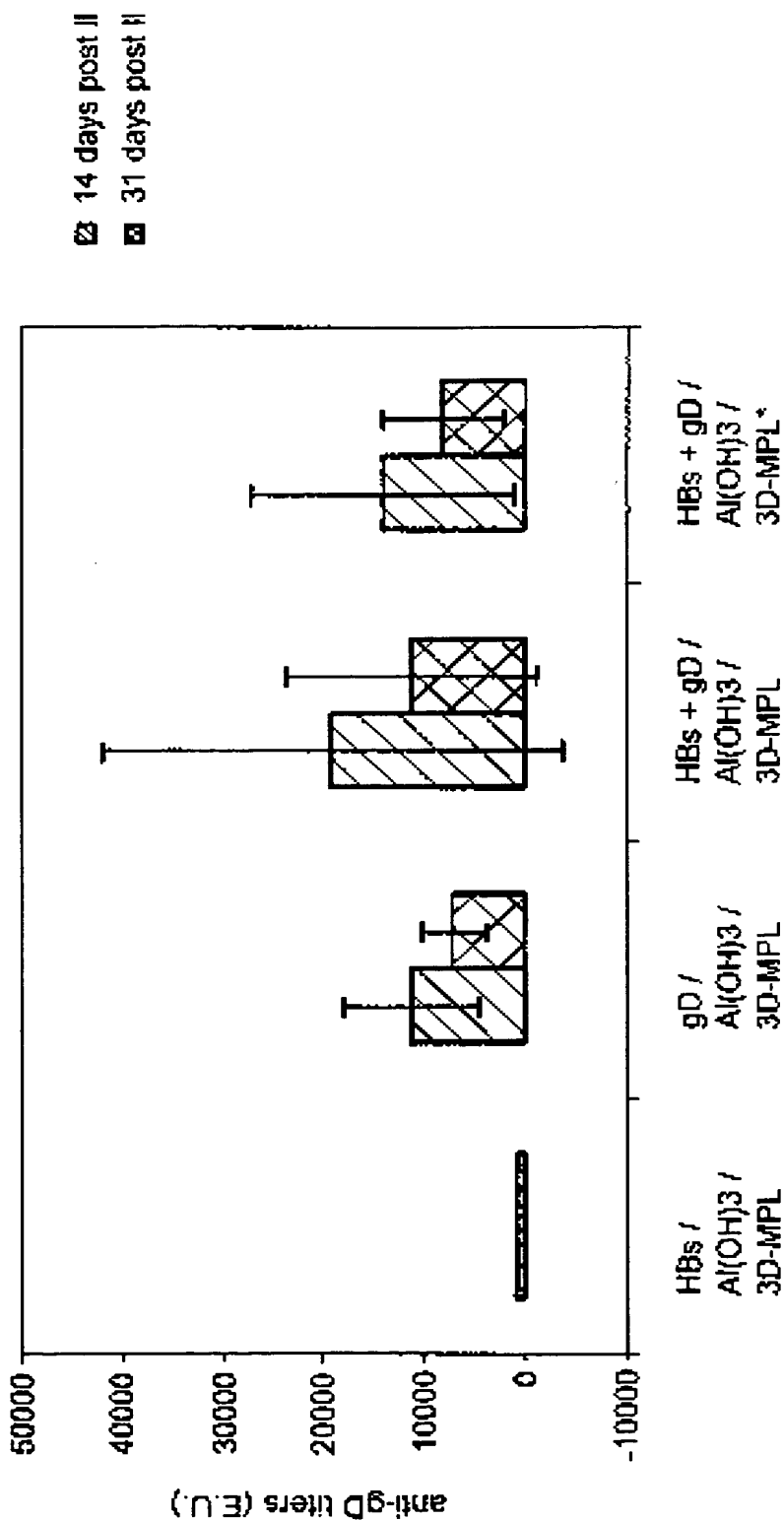


Fig. 7

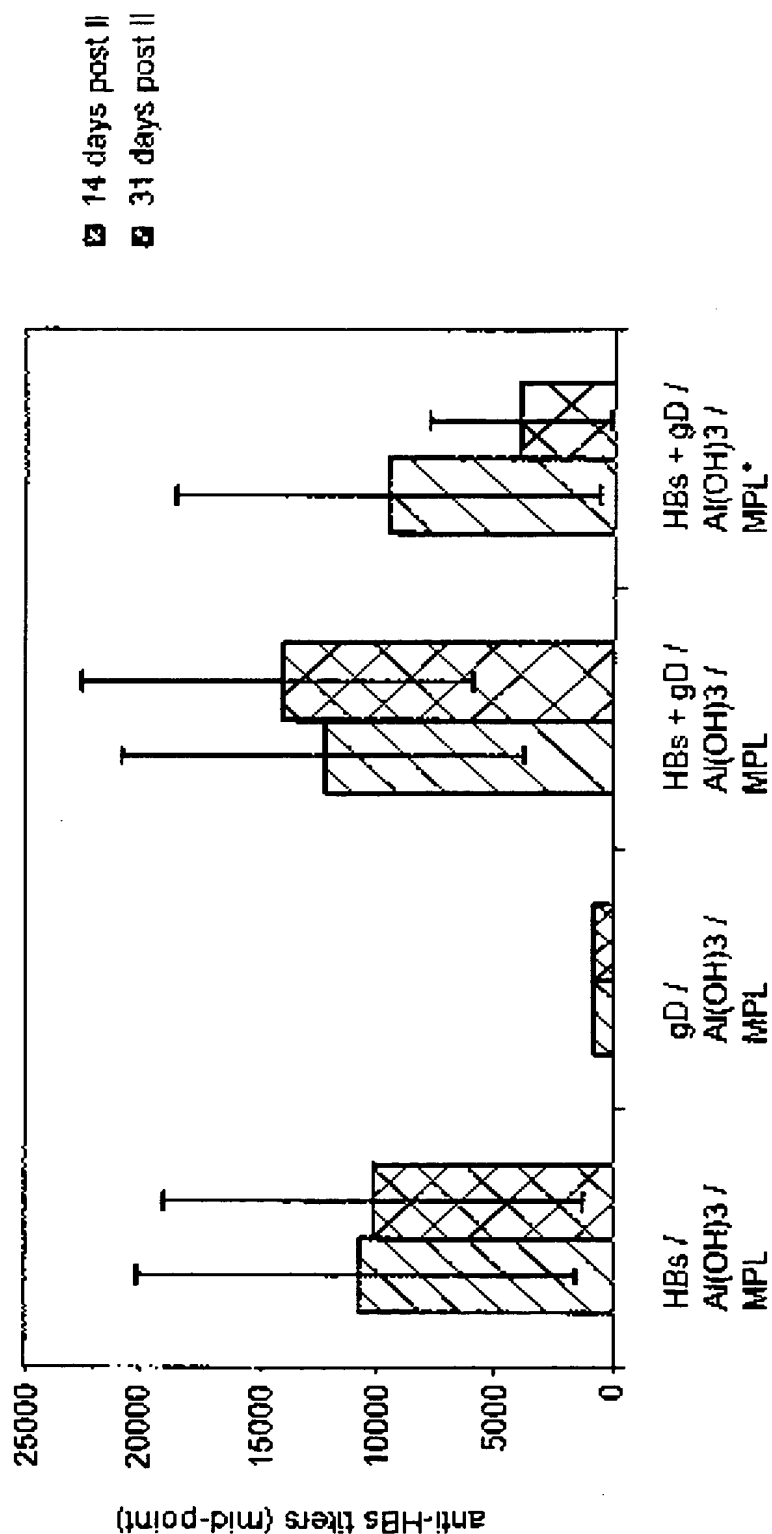
Similar number of animals had recurrences in the gD + HBs and gD alone groups. Exactly the same number of animals in these groups had more than 1 recurrence during the observation period (day 13 to 39 post challenge). In those animals with recurrences, comparable lesion severity were recorded.





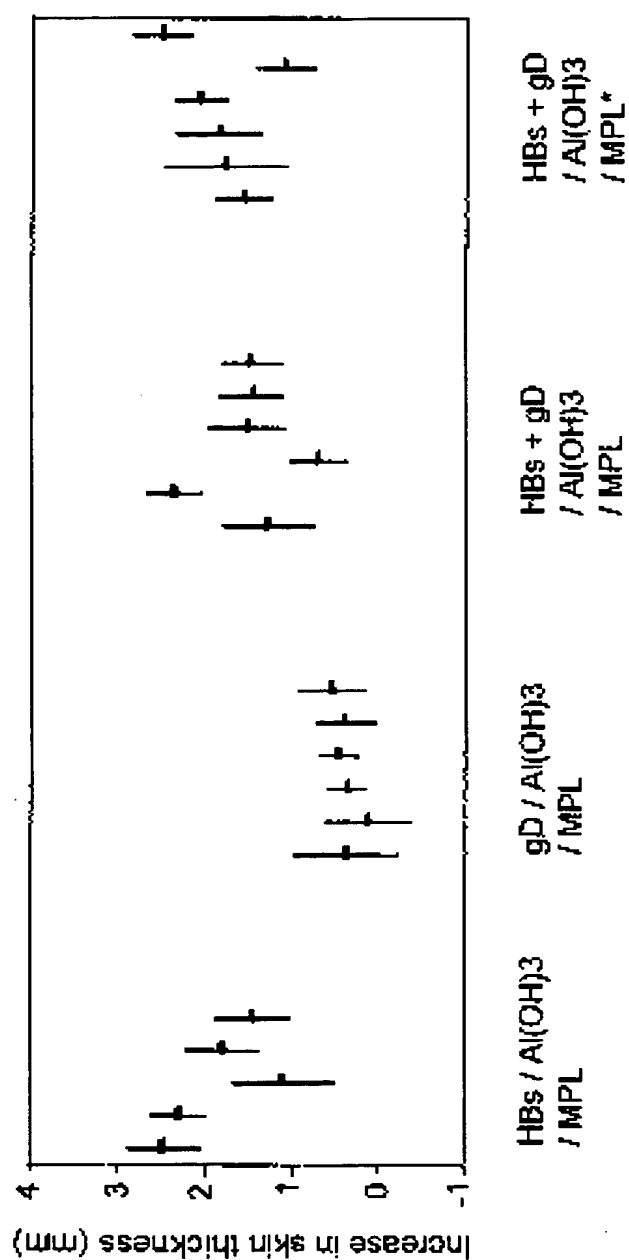


**Fig. 1** anti-gD ELISA titers

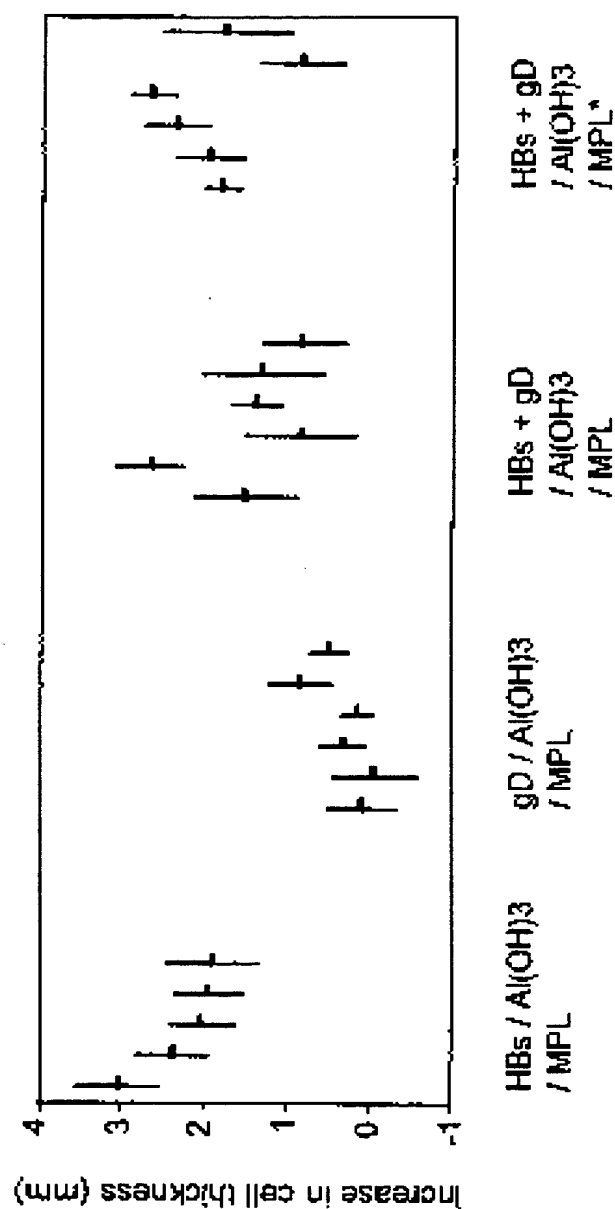


**Fig. 2** anti-HBs ELISA titers

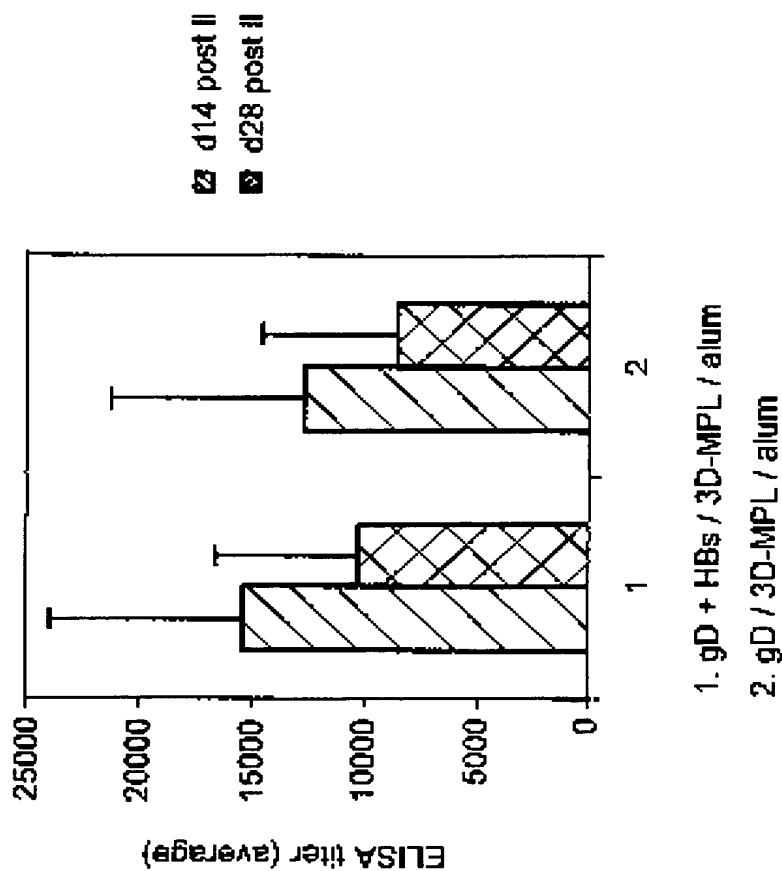
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**Fig. 3** DTH reaction 24 hrs

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**Fig. 4** DTH reaction at 48 hrs

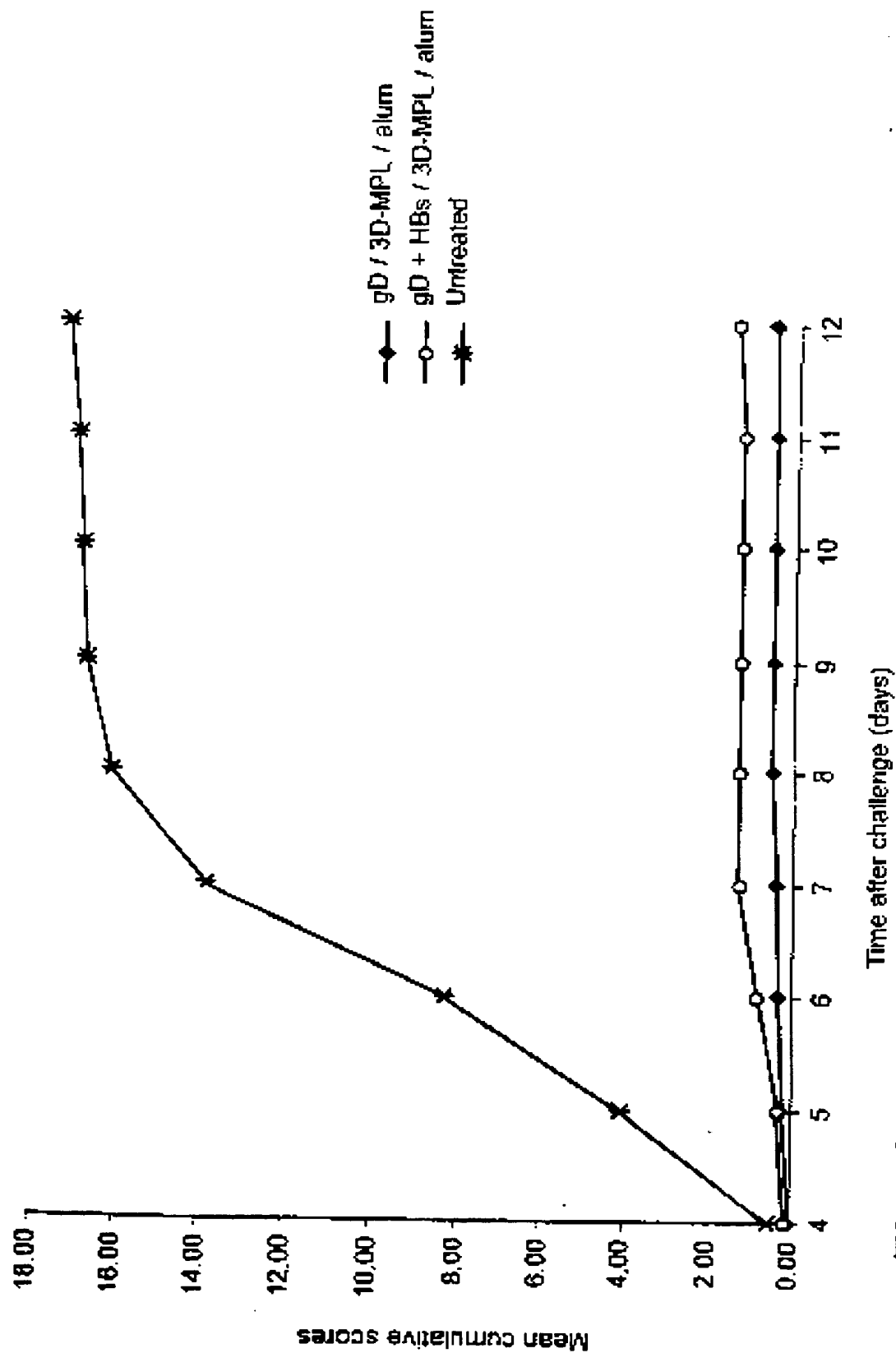
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**Fig. 5**

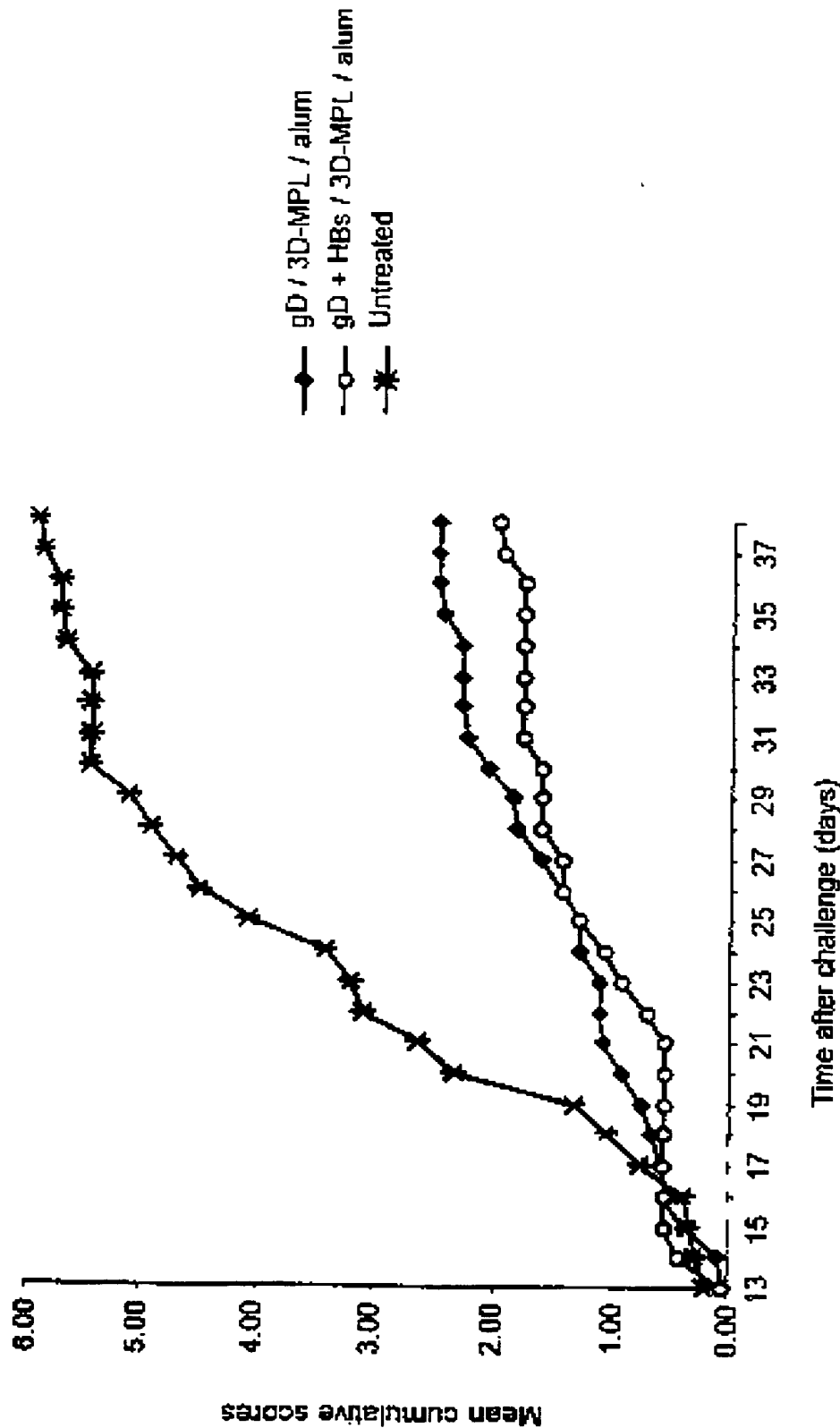
Similar ELISA and neutralizing titers were induced by the gD (5µg) + HBs (5µg) combination in 3D-MPL / alum and gD / 3D-MPL / alum.

6/7

**Fig. 6**

Both guinea pigs immunized with the gD + HBs combination and guinea pigs immunized with gD alone were very well protected against HSV challenge. While most control animals had lesions, these were observed in respectively 2 / 12 and 3 / 12 animals only in the gD + HBs and gD alone groups.

7/7

**Fig. 7**

Similar number of animals had recurrences in the gD + HBs and gD alone groups. Exactly the same number of animals in these groups had more than 1 recurrence during the observation period (day 13 to 39 post challenge). In those animals with recurrences, comparable lesion severities were recorded.





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<b>(21) International Application Number:</b> PCT/EP99/01406 <b>(22) International Filing Date:</b> 4 March 1999 (04.03.99)  <b>(30) Priority Data:</b> 9805105.5 9 March 1998 (09.03.98) GB 9813561.9 23 June 1998 (23.06.98) GB  <b>(71) Applicant (for all designated States except US):</b> SMITHKLINE BEECHAM BIOLOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> STEPHENNE, Jean [BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). WETTENDORFF, Martine, Anne, Cecile [BE/BE]; SmithKline Beecham Biologicals s.a., Rue de l'Institut 89, B-1330 Rixensart (BE).  <b>(74) Agent:</b> TYRRELL, Arthur, William, Russell; SmithKline Beecham Corporate Intellectual Property, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>  <b>(88) Date of publication of the international search report:</b> 25 November 1999 (25.11.99)
<b>(54) Title:</b> COMBINED VACCINE COMPOSITIONS  <b>(57) Abstract</b>  Novel combined vaccine compositions preferentially for administration to adolescents are provided, comprising a hepatitis B viral antigen and a herpes simplex viral antigen and optionally in addition one or more of the following: an EBV antigen, a hepatitis A antigen or inactivated attenuated virus, an HPV antigen, a VZV antigen, an HCMV antigen, a Toxoplasma gondii antigen. The vaccine compositions are formulated with an adjuvant which is a preferential stimulator of TH1 cell response such as 3D-MPL and QS21.		

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# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/EP/92/01406

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 A61K39/295 A61K39/39

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 11291 A (SMITHKLINE BEECHAM BIOLOG) 9 July 1992 (1992-07-09) page 1, line 3 - line 6; claims 1,3,9,14 page 1, line 15 - line 33 page 2, line 19 - line 24 page 3, line 5 - line 36 page 8, line 20 - line 30 page 12, line 23 - line 27 page 13, line 16 - line 24 ---	1-6, 20
A	EP 0 510 996 A (UNIV OSAKA RES FOUND) 28 October 1992 (1992-10-28) page 2, line 1 - line 4; claims 1,9,13 page 2, line 58 -page 3, line 23 page 3, line 35 - line 45 --- -/--	1,14,15, 20

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents :

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Date of the actual completion of the international search

12 October 1999

Date of mailing of the international search report

19/10/1999

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 99/01406

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 339 667 A (CHEMO SERO THERAPEUT RES INST) 2 November 1989 (1989-11-02) cited in the application page 2, line 1 - line 10; claim 1 -----	1,9,20

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